

## The effect of copper fungicides on the rate of photosynthesis and the transpiration of hop plants

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**Key words:** hop, photosynthesis, transpiration, copper, Agnus

### SUMMARY

*The paper evaluates the effect of copper fungicide spraying on the rate of photosynthesis and transpiration of hops, the influence of spraying on the elemental copper content in the leaves and cones hop variety Agnus. Photosynthetic rate was measured by LC pro+ (infrared analyzer) in the Hop Research Institute Saaz in the field in some periods of 2008, 2009 and 2010. Dry cones and leaf samples (taken before and after application of copper fungicides) were analyzed in an accredited laboratory for elemental copper.*

### INTRODUCTION

Photosynthetic reaction, the importance of which is based on energy conversion of sunlight into energy of chemical bonds, is one of the most important physiological processes in plants. Solar radiation is not fully utilized by plants, because the energy hitting the earth is absorbed by only 0.8%. The use of solar energy ranges from 1.0 to 2.5% for the whole growing season of agricultural crops. The basic metabolic process in hop plants is carbon metabolism, both the active part - photosynthesis, and the passive part – respiration (Strasil, 1998). The photosynthetic process of hops begins at the beginning of the hop sprouting. Photosynthetic rate gradually increases in the next period. The active balance of photosynthesis and respiration processes occurs through elongation growth and the thickening of individual plant organs. Dependence on photosynthesis has several causes at the level of development. Very young leaves still below full leaf area and thus capture smaller amounts of radiation have less chlorophyll and breathe heavily. Two significant changes in enzyme activity occur in further stages of the plant's development, especially in the transition from vegetative to generative phase. The photosynthetic capacity is high and very important during flowering and fruit formation phase for this reason (Rybáček et al., 1980). All aboveground organs of hop plants are ready for intensive course of photosynthesis expect fruit. The main importance, however, have hopvine and leaves. A higher photosynthetic capacity show lateral branches leaves than hopvine leaves. The differences in the course of photosynthesis are also interesting within the frame of leaf blade. A higher photosynthetic capacity is in the middle of the blade over the edges, top and base of the sheets. It is also interesting relatively high photosynthetic rate of old hopvine and yellowing leaves. The cones reach full intensity of photosynthesis after flowering (Larcher, 1995). To study the photosynthesis production of higher plants, gasometry methods are mainly used. Applying these methods monitors changes of CO<sub>2</sub> concentrations in the vicinity of the measured plant part or the rate of decrease in the concentration of CO<sub>2</sub> in the air to which the plant is exposed, where necessary. When measuring, the leaf, plant or the part of the plant is enclosed in an air-conditioned assimilation chamber under constant conditions of temperature, humidity, radiation and CO<sub>2</sub> concentration (Sestak, Catsky, 1966).

The European Union has limited the use of synthetic fungicides in plant protection. Therefore, more attention is paid to the use of inorganic salts against mycoses (Glendinning, 2009). Among other substances also copper are used in the plant protection of hops such as: copper hydroxide, copper and other oxochlorid formulation of copper. These compounds are toxic to certain groups of fungi and bacteria. The most serious fungal disease of hop from the group of true fungi is particularly downy mildew (*Pseudoperonospora humuli*), against which must be chemically treated several times for vegetation (Hluchý a kol., 2008). Copper fungicides (e.g., Kuprikol 50, Kuprikol 250 SC, Cuproxat CS, Curzate K) are an important regulatory factor in the occurrence of downy mildew (*Pseudoperonospora humuli* L.) in the period of flowering and formation of hop cones. Copper is needed for normal growth and development of plants, but is also potentially toxic. Lack or excess of copper can cause disturbances in plant growth and development of the negative impact on physiological processes in plants (Yruela, 2005). The excessive copper in the cells slows growth and important cellular processes, it can cause oxidative stress in plants and consequently increased production of toxic oxygen free radicals (Luna et al., 1994; Stohs and Bagchi, 1995).

The aim of the experiment was to monitor and compare the effects of multiple spraying (untreated, one spray treatment, two or three spray treatment) of copper fungicides on photosynthetic activity and transpiration of hop plants and to monitor elemental copper in hops (in leaves and hop cones) after repeated applications of copper in an integrated system of hops growing.

## MATERIALS AND METHODS

The experimental hop garden is located in the Hop Research Institute in Saaz at an altitude of 215 m in the Saaz hop growing region and Poohří central location, warm and dry region, the sum of temperatures above 10 °C is 2600-2800 °C per year. Consumptive water security is in the range 0 – 2. Dry growing season is 40-60 days. The average annual air temperature ranges from 8-9 °C, average annual rainfall is below 500 mm. Exposure of the land is flat and omnidirectional. It is planted by high alpha variety Agnus and equipped with drip irrigation.

The technology of hop growing encompassed the normal duties used in the production of hop gardens.

We aimed to measure important physiological parameters (photosynthesis and transpiration rate) as response to stressful conditions caused by multiple treatments of hops by copper (Kuprikol 50, Kuprikol 250 SC, Cuproxat CS, Curzate K).

Specific application terms and appropriate doses of elemental copper in each treatment expressed in kg/ha during the experimental period are shown in *Table 1*. Physiological processes were measured by LC pro+ device allows for the measurement and monitoring of the basic physiological processes in the leaf without its separation from the plant.

The hop garden (10244 m<sup>2</sup>) was divided into blocks I, II., III., according to variants of cupric fungicide treatment. In the first block I. the copper was applied only - 1x, in the second block II. - 2x and in the third block - 3x in 2008. The first block I. was without treatment of copper, in the second block II, the copper was applied 1x and the third block III. was with 2 applications of copper fungicides in 2009 and again in 2010. 2 or 4 average plants were chosen approximately in the same stage of growth from each variant. The hop plants were identified and physiological processes were measured in the height of about 3 m on the lateral branches leaves. The optimum temperature of 23 °C was chosen in the measuring chamber of LC pro+ device. The density of irradiation was set at 600 nm.

Individual measurements were before applications and at weekly intervals after the applications of copper fungicides in 2008, 2009 and 2010 (see *Table 2, 3 a 4*).

Measurements were made at approximately the same hour ± 30 minutes. The values of measurements were automatically recorded after intervals of 1 min. after stabilization of conditions inside the measuring chamber.

Furthermore, during the growing seasons of 2008-2010 the tests were carried out after repeated application of copper fungicide to determine the effects of elemental copper in hops (leaves, cones) and follow the evolution time of the flowering stage respectively the beginning of formation of hop cones to harvest. The leaves and cones were sampled at random from 10 plants in high-rise floors 2 to 6 meters in the dates indicated in *Tables 5-7*.

Dry samples of cones and leaves were analyzed by a qualified laboratory for elemental copper. The results are summarized in *Tables 5 to 7*.

Table 1

The terms of application of copper fungicides and doses of elemental copper (Cu kg/ha)

treatments	2008		terms of applications	2009		2010
	terms of applications	dose Cu (kg/ha)		dose Cu (kg/ha)	terms of applications	dose Cu (kg/ha)
I.	<b>21.7.</b>	8,9	<b>27.7.</b>	5	<b>13.7.</b>	2,8
II.	<b>12.8.</b>	8,9	<b>14.8.</b>	2,9	<b>1.8.</b>	5
II.	<b>27.8.</b>	8,9	-	-	<b>22.8.</b>	5

## RESULTS A DISCUSSION

The values of photosynthetic rate ranged from 5.22 to 8.73 (see *table 4*) over the period of 2010. The rate of photosynthesis increased significantly (about 1.96 and 2.5  $\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$ ) in 2010 in treated variants after the first application of copper fungicide (1.8.) (see *figure 3*). The rate of photosynthesis remained essentially constant in hop plants of control variant. The rate of photosynthesis also increased after application of copper fungicides in 2008 and 2009, but also in untreated control (*Figure 1 and 3*). The increase of the values associated with the course of weather conditions. The increase of the rate photosynthesis values associated with the course of weather conditions. The downward trend is similar in values of photosynthetic rate over a long period from the date of spraying in all the years 2008, 2009 and 2010. The hop plant receives after the stressful period to the normal level of production capacity, corresponding to actual conditions for growth and development.

Table 2

**The rate of photosynthesis and transpiration after application of copper fungicides in 2008**

Terms of measurements	Characteristic of variations	photosynthesis rate ( $\mu\text{mol CO}_2 \text{ m}^{-2} \cdot \text{s}^{-1}$ )			transpiration rate ( $\text{mmol H}_2\text{O m}^{-2} \cdot \text{s}^{-1}$ )		
		I.block	II.block	III.block	I.block	II.block	III.block
<b>16.7.</b>	before spraying	5,17	5,57	6,3	1,04	1	1,03
<b>24.7.</b>	after the first spraying	7,37	7,07	7,89	1,05	1,06	1,13
<b>6.8.</b>	after the first spraying	5,32	5,05	4,56	0,94	0,83	0,81
<b>14.8.</b>	after the second spraying	5,66	4,99	6,95	0,93	0,89	1,09
<b>20.8.</b>	after the second spraying	6,11	7,05	7,07	1,05	1,06	1,08
<b>27.8.</b>	after the second spraying	7,55	7,01	7,69	1,18	0,97	0,92
<b>3.9.</b>	after the third spraying	6,36	6,25	7,99	1,05	0,86	1,05
<b>average</b>		<b>6,22</b>	<b>6,14</b>	<b>6,92</b>	<b>1,03</b>	<b>0,95</b>	<b>1,02</b>

The value of the rate of photosynthesis increased slightly in plants (measuring 23.8.), where copper was applied twice - III. block of hop garden (the application on August 1 and August 22) (value increased by  $0,18 \mu\text{mol CO}_2 \text{ m}^{-2} \cdot \text{s}^{-1}$ ). The photosynthesis increased significantly at variant with one copper treatment. The values of photosynthesis were essentially constant in the untreated controls again in this time of measuring. The highest photosynthetic rate was during the reporting period in the block II. on average ( $7,29 \mu\text{mol CO}_2 \text{ m}^{-2} \cdot \text{s}^{-1}$ ).

Table 3

**The rate of photosynthesis and transpiration after application of copper fungicides in 2009**

Terms of measurements	Characteristic of variations	photosynthesis rate ( $\mu\text{mol CO}_2 \text{ m}^{-2} \cdot \text{s}^{-1}$ )			transpiration rate ( $\text{mmol H}_2\text{O m}^{-2} \cdot \text{s}^{-1}$ )		
		I.block	II.block	III.block	I.block	II.block	III.block
<b>17.6.</b>	before spraying	8,08	3,14	6,48	1,37	0,92	0,99
<b>24.6.</b>	before spraying	8,37	5,94	8,16	1,19	1,2	1,28
<b>15.7.</b>	before spraying	4,65	3,72	5,27	0,91	0,83	1,08
<b>23.7.</b>	before spraying	2,84	4,56	5,91	0,53	0,64	0,61
<b>30.7.</b>	after the first spraying	5,31	4,04	5,84	1,04	0,67	0,84
<b>5.8.</b>	after the first spraying	5,81	3,82	5,07	0,79	0,64	0,78
<b>12.8.</b>	after the first spraying	5,67	3,67	5,03	0,87	0,68	0,74
<b>20.8.</b>	after the second spraying	6,62	6,33	5,32	1,09	0,85	0,77
<b>26.8.</b>	after the second spraying	4,16	4,44	4,42	0,98	1,04	0,79
<b>average</b>		<b>5,65</b>	<b>4,58</b>	<b>5,72</b>	<b>0,97</b>	<b>0,85</b>	<b>0,88</b>

The photosynthesis increased significantly after the first application of copper in 2010, it is the same situation as in 2008 but the results were different in 2009 (see *figure 1 and 3*). The photosynthesis increased after the first application of copper in this case, but slightly. The values of photosynthetic rates reduced after 10 days before application in variants with spray again as in 2008 and 2009. The hop plants is getting to the resting phase after the stressful period.

Table 4

**The rate of photosynthesis and transpiration after application of copper fungicides in 2010**

Terms of measurements	Characteristic of variations	photosynthesis rate ( $\mu\text{mol CO}_2 \text{ m}^{-2} \cdot \text{s}^{-1}$ )			transpiration rate ( $\text{mmol H}_2\text{O m}^{-2} \cdot \text{s}^{-1}$ )		
		I.block	II.block	III.block	I.block	II.block	III.block
<b>22.7.</b>	before spraying	5,38	5,49	5,29	0,65	0,55	0,56
<b>28.7.</b>	before spraying	5,22	6,29	5,92	0,73	0,97	0,84
<b>4.8.</b>	after the first spraying	5,43	8,25	8,42	0,79	1,08	1
<b>11.8.</b>	after the first spraying	5,33	7,01	6,22	0,87	0,96	0,74
<b>17.8.</b>	after the first spraying	6,8	7,85	8,03	1,09	1,2	1,21
<b>23.8.</b>	after the second spraying	6,84	8,73	8,21	1,05	0,99	1,01
<b>2.9.</b>	after the second spraying	5,32	7,42	3,81	0,86	0,95	0,75
<b>average</b>		<b>5,76</b>	<b>7,29</b>	<b>6,56</b>	<b>0,86</b>	<b>0,96</b>	<b>0,87</b>

The transpiration rate was more balanced than the rate of photosynthesis in 2010 as well as in the years 2008, 2009. The measurement values for the monitored years ranged between  $0,55$  do  $1,37 \text{ mmol H}_2\text{O m}^{-2} \cdot \text{s}^{-1}$  (see *table 1, 2 and 3*). The lowest value was measured in the first term (July 22, 2010) -  $0,55 \text{ mmol H}_2\text{O m}^{-2} \cdot \text{s}^{-1}$ . We can say

that the highest average value of transpiration was in the II. block of hop garden in 2010 ( $0,96 \text{ mmol H}_2\text{O m}^{-2}\text{s}^{-1}$ ). The average values was similarly for other blocks of hop plants ( $0,86$  and  $0,87 \text{ mmol H}_2\text{O m}^{-2}\text{s}^{-1}$ ).

The average transpiration rate ranged among  $0,95 - 1,03 \text{ mmol H}_2\text{O m}^{-2}\text{s}^{-1}$ , respectively  $0,85 - 0,97 \text{ mmol H}_2\text{O m}^{-2}\text{s}^{-1}$  in 2008 and 2009.

The following conclusions obtained from the results:

- ❖ the elevation occurred in activity of the hop plants after treatment in 2008, 2009 and 2010.
- ❖ the foliar application of copper not reduced the photosynthesis rate
- ❖ the foliar application of copper not reduced the transpiration rate

The gradual decline of copper content dominated especially in leaves with running time (see *table 5, 6 and 7*). In the case of hop cones this trend is not so significant (see *table 5, 6 and 7*).

The most of copper was applied in an attempt in 2008 ( $3 \times 8.9 \text{ kg}$ ). The copper content in the hop cones  $471 \text{ mg/kg}$  was found during the harvest. The comparable content was detected in an attempt of 2009. The copper content in hop cones after the three applications of copper fungicides ( $12.8 \text{ kg Cu/ha}$ ) was only  $323 \text{ mg/kg}$  in 2010.

The following conclusions obtained from the results:

- ❖ there is no clear correlation between the quantity of copper applied in the crops chemical treatment with resulting of copper content in hop cones during the harvest
- ❖ the maximum content of Cu are well below the limit of  $1000 \text{ mg/kg}$  after repeated applications of copper fungicides.

The much higher copper content were found in the hop leaves as expected. The final content in the range  $1000-2000 \text{ mg/kg}$  were detected in hop leaves after repeated spraying of fungicides. The experimental results (in 2009) showed that the natural biological background of copper content in hop leaves and cones is less than  $20 \text{ mg/kg}$ .

## CONCLUSION

The three-year results evaluating the effect of antifungal treatment with cupric to the rate of photosynthesis and transpiration shows that in 2008, 2009 and 2010, after treatment, the activity of the plant relative increase. The values of transpiration rates are the reaction to the current state of hop growth and environment. The application of copper on hop leaves does not lessen photosynthesis and transpiration of hop plants.

There is no clear correlation between the quantities of copper applied in the crops chemical treatment with resulting in copper content during the harvest. The copper content of hop cones well below the limit of  $1000 \text{ mg/kg}$  after repeated applications of copper fungicides.

Table 5

The copper content in the leaves and hop cones after the application of copper fungicides in 2008

sampling day	time after application (days)			Cu content (mg/kg)				in leaves ***	in hop cones***
	after the first spraying	after the second spraying	after the third spraying	in leaves*	in hop cones*	in leaves**	in hop cones**		
15.7.	untreated control			227					
22.7.	5			1778					
24.7.	7			1918					
28.7.	11			1580					
31.7.	14			1316					
8.8.	22			1566	405				
13.8.	27	1		924		2508			
14.8.	28	2		843	201	1557	504		
18.8.	32	6		746	133	2091	487		
25.8.	39	13		835	122	1686	309		
28.8.	42	16	1	726	93	1115	247	2637	834
1.9.	46	20	5	665	86	823	143	2267	705
5.9.	50	24	9	694	108	770	201	1814	471

\*after the first spraying of the copper fungicides

\*\*after the second spraying of the copper fungicides

\*\*\*after the third spraying of the copper fungicides

Table 6

**The copper content in the leaves and hop cones after the application of copper fungicides in 2009**

sampling day	time after application (days)		Cu content (mg/kg)			
	after the first spraying	after the second spraying	in leaves*	in hop cones*	in leaves**	in hop cones**
	untreated control		15	6		
27.7.	2h.		1010	371		
28.7.	1		957	302		
31.7.	4		892	286		
3.8.	7		900	395		
6.8.	10		888	417		
11.8.	15		878	335		
14.8.	18	2h.			1175	489
17.8.	21	3	744	415	1027	501
21.8.	25	7			1269	556
24.8.	28	11	711	429		
28.8.	32	14	479	350	1094	456

\* after the first spraying of the copper fungicides

\*\* after the second spraying of the copper fungicides

Table 7

**The copper content in the leaves and hop cones after the application of copper fungicides in 2010**

sampling day	time after application (days)		Cu content (mg/kg)			
	after the first spraying	after the second spraying	in leaves*	in hop cones*	in leaves**	in hop cones**
	untreated control		383	109		
2.8.	1		1598	312		
4.8.	3		1442	316		
8.8.	7		1599	219		
16.8.	14		1425	176		
23.8.	22	1	1151	208	2021	482
30.8.	29	8	1071	175	2148	505
6.9.	36	15	1100	153	2396	491
10.9.	40	19	1281	144	1698	323

\* after the first spraying of the copper fungicides

\*\* after the second spraying of the copper fungicides

Figure 1. The course of photosynthetic rate ( $\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$ ) of hop plants treated with cupric fungicides in 2008

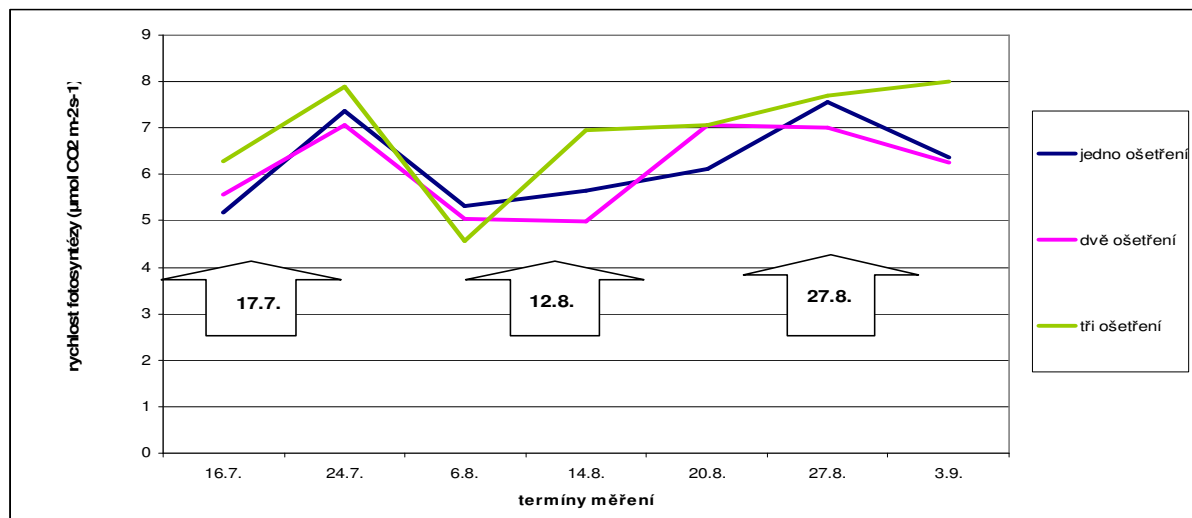


Figure 2. The course of photosynthetic rate ( $\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$ ) of hop plants treated with cupric fungicides in 2009

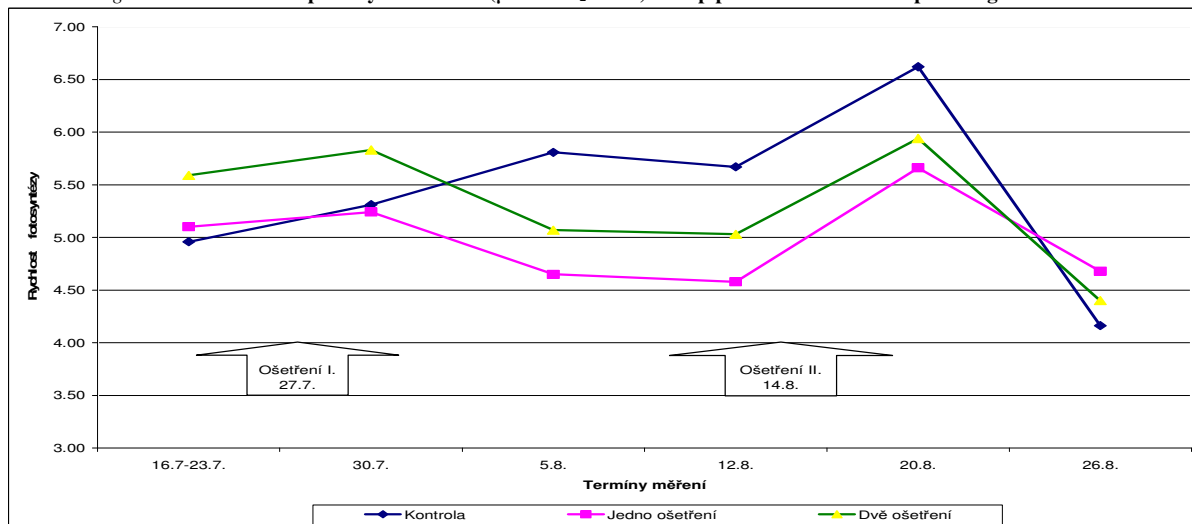
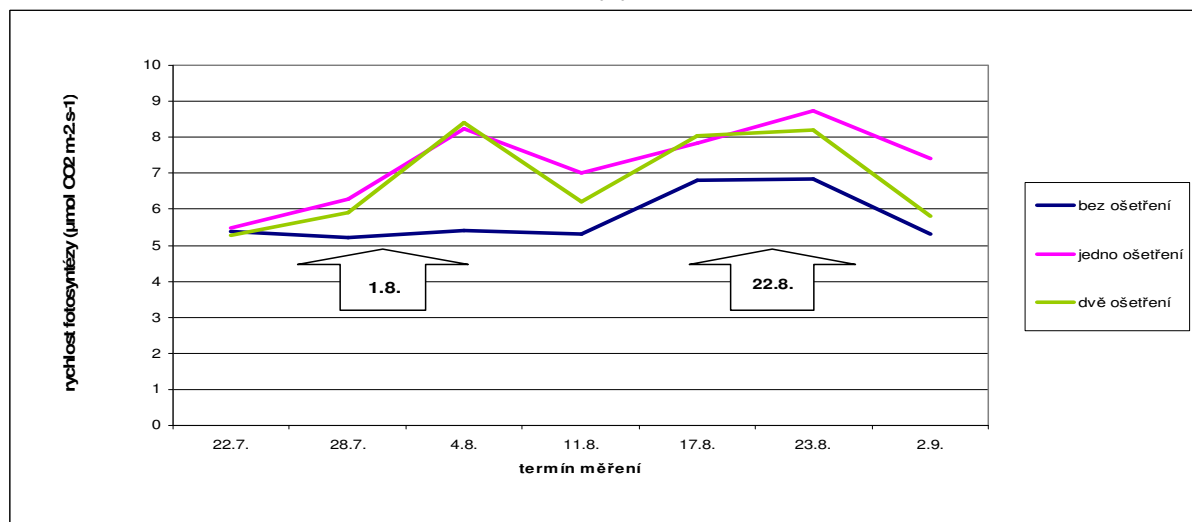


Figure 3. The course of photosynthetic rate ( $\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$ ) of hop plants treated with cupric fungicides in 2010



## **INSCRIPTION**

Acknowledgements: Supported by the Ministry of Education, Youth and Sports of the CR Research Plan No. MSM. 6046070901 and the Ministry of Agriculture of the CR NAZV Project No. QH 81049/2008.

## **REFERENCES**

- Glendinning, P.(2009): Ekologicky příznivější možnosti ochrany chmele proti škodlivým organismům. *Chmelařství*, 2009, 56 (4), s. 32-33.
- Hluchý, M.-Ackermann, P.-Zacharda, M.-Laštůvka, Z.-Bagar, M.-Jetmarová, E.-Vanek, G.-Szöke, L.-Plíšek, B.(2008): Ochrana ovocných dřevin a révy v ekologické a integrované produkci. *Biocont Laboratory*, Brno, 2008, s. 498
- Larcher, W.-Biederman, M.-Thorson, A.(1995): *Physiological plant ecology*. 1995, 3. vydání. Berlín Springer, s. 303
- Luna, C. M.-Gonzalez, C. A.-Trippi, V. S.(1994): Oxidative damage caused by an excess of copper in oat leaves. *Plant and Cell Physiology*, 35 (1), 1994, s. 11 – 15.
- Rybáček V. (1980): *Chmelařství*. Praha: SZN Praha, ISBN 07-068-80-04/37, 1980, s. 426
- Stohs, S. J.-Bagchi, D.(1995): Oxidative mechanisms in the toxicity of metal ions. *Free Radical Biology & Medicine*, 18 (2), 1995, s. 321 – 336
- Stražil, Z. (1998): Využití kalorimetrického měření pro potřeby rostlinné výroby. In: *Mezinárodní slovenský a český kalorimetrický seminář* (Taraba ed.), 1998, s. 39-40.
- Šesták Z.-Čatský J. (1966): *Metody studia fotosyntetické produkce rostlin*. Academia, Praha, 1966, s. 394
- Yruela, I. (2005): Copper in plants. *Brazilian Journal of Plant Physiology*, 17 (1), Londrina, 2005